

SEQUENCE LISTING

<110> Chauhan, Sarita
DiCosimo, Robert
Payne, Mark
Gavagan, John
Fallon, Robert

<120> Isolation and Expression of a Gene for Nitrilase from
Acidovorax Facilis 72W

<130> BC-1032 US NA

<140>
<141>

<150> 60/193,707
<151> March 31, 2000

<160> 32

<170> Microsoft Office 97

<210> 1
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Forward primer
(1F)

<220>
<223> K= G or T, M= A or C, S= G or C, Y= C or T

<400> 1
tkkmtkccsg gctaycc
17

<210> 2
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Reverse primer
(7R)

<220>
<223> S= G or C, H= A or C or T, M= A or C, R= A or G,
Y= C or T

<400> 2
ggccasshtg mrayrtg
17

<210> 3
<211> 385
<212> DNA
<213> Acidovorax facilis

<400> 3
ctattgggcg tggctcggcg acgtgaagta cagcctaagc tttacttcac gctatcacga
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gaattcgttg gagctaggtg acgaccgtat gcgtcgcctc cagctggccg cgcgcgcgcaa
120
caaaatcgca ctctgcatgg gctattcgga gcggaagcc ggatcgcgct atctgagcca
180
ggtgttcacg gacgagcgtg gcgagatcgt tgccaatcgg cgcaagctga agccacaca
240
cgttgagcgt acgatctacg gcgaaggcaa cggaaccgat ttcctcacgc acgacttcgc
300
gttcggacgc gtcggtggat tgaactgctg ggaacatttc caaccgctca gcaagttcat
360
gatgtacagc ctcggtgagc aggtc
385

<210> 4
<211> 1110
<212> DNA
<213> Acidovorax facilis

<400> 4
gtggtttcgt ataacagcaa gttcctcgcg gcaaccgttc aggcagagcc ggtatggctc
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gacgcagacg caacgatcga caagtcgatc ggcatcatcg aagaagctgc caaaagggc
120
gcgagtctga tcgctttccc ggaagtattc attccgggct acccctattg ggcgtggctc
180
ggcgacgtga agtacagcct aagctttact tcacgctatc acgagaattc gttggagcta
240
ggtgacgacc gtatgcgtcg cctccagctg gccgcgcgcc gcaacaaaat cgcactcgtc
300
atgggctatt cggagcggga agccggatcg cgctatctga gccagggtgt catcgacgag
360
cgtggcgaga tcgttgccaa tcggcgcaag ctgaagccca cacacgttga gcgtacgatc
420
tacggcgaag gcaacggaac cgatttcctc acgcacgact tcgcgttcgg acgcgtcggc
480
ggattgaact gctgggaaca tttccaaccg ctacgcaagt tcatgatgta cagcctcggc
540
gagcaggtcc acgttgcatc gtggccggcg atgtcccctc ttcagccgga tgttttccaa
600
ctgagcatcg aagccaacgc gacggtcacc cgctcgtacg caatcgaagg ccaaaccctt
660
gtgctttgct cgacgcaggt gatcggacct agcgcgatcg aaacgttctg cctcaacgac
720
gaacagcgcg cactgttgcc gcaaggatgt ggctgggccc gcatttacgg cccggatgga
780
agcgagcttg cgaagcctct ggcggaagat gctgagggga tcttgtagcg agagatcgat
840
ctggagcaga ttctgctggc gaaggctgga gccgatccgg tcgggcacta ttcgcggcct
900
gacgtgctgt cggccagtt cgaccgcgcg aatcatacgc cagttcatcg catcggcatt
960
gacggctcgt tggatgtgaa taccgcagc cgcggtggaga atttccgact gcgacaagcg
1020
gctgagcagg agcgtcaggc atccaagcgg ctcggaacga aactctttga acaatccctt
1080
ctggctgaag aaccgggtccc agcaaagtag
1110

<210> 5

[illegible]

3

Asp Gly Arg Leu Asp Val Asn Thr Arg Ser Arg Val Glu Asn Phe Arg
 325 330 335

Leu Arg Gln Ala Ala Glu Gln Glu Arg Gln Ala Ser Lys Arg Leu Gly
 340 345 350

Thr Lys Leu Phe Glu Gln Ser Leu Leu Ala Glu Glu Pro Val Pro Ala
 355 360 365

Lys

<210> 6
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Primer

<400> 6
 gacgcatatg gtttcgtata acagcaa
 27

<210> 7
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Primer

<400> 7
 cgacggatcc ttatggctac tttgctgg
 28

<210> 8
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Primer

<400> 8
 cggatccatg gtttcgtata acagcaagtt c
 31

<210> 9
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Primer

<400> 9
 ttatggctac tttgctggga ccg
 23

<210> 10
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 10
tacatatggt ttcgtataac agcaagttc
29

<210> 11
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 11
catctcgaga tggtttcgta taacag
26

<210> 12
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 12
cactcgagct actttgctgg gac
23

<210> 13
<211> 1110
<212> DNA
<213> Acidovorax facilis

<400> 13
atgggttcgt ataacagcaa gttcctcgcg gcaaccgttc aggcagagcc ggtatggctc
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gacgcagacg caacgatcga caagtcgatc ggcacatcgc aagaagctgc ccaaaagggc
120
gcgagtctga tcgctttccc ggaagtattc attccgggct acccctattg ggcgtggctc
180
ggcgacgtga agtacagcct aagctttact tcacgctatc acgagaattc gttggagcta
240
ggtgacgacc gtatgcgtcg cctccagctg gccgcgcgcc gcaacaaaat cgcactcgtc
300
atgggctatt cggagcggga agccggatcg cgctatctga gccagggtgtt catcgacgag
360
cgtggcgaga tcgttgccaa tcggcgcaag ctgaagccca cacacgttga gcgtacgac
420
tacggcgaag gcaacggaac cgatttcctc acgcacgact tcgcgttcgg acgcgtcggt
480

ggattgaact gctgggaaca tttccaaccg ctcagcaagt tcatgatgta cagcctcggg
 540
 gagcaggtcc acgttgcatc gtggccggcg atgtccctc ttcagccgga tjttttccaa
 600
 ctgagcatcg aagccaacgc gacggtcacc cgctcgtacg caatcgaagg ccaaaccttt
 660
 gtgctttgct cgacgcaggt gatcggacct agcgcgatcg aaacgttctg cttcaacgac
 720
 gaacagcgcg cactgttgcc gcaaggatgt ggctgggcgc gcatttacgg ccgggatgga
 780
 agcgagcttg cgaagcctct ggcggaagat gctgagggga tcttgtagcg agagatcgat
 840
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 900
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 960
 gacggtcgct tggatgtgaa taccgcaggt cgctgggaga atttccgact ggcacaagcg
 1020
 gctgagcagg agcgtcaggc atccaagcgg ctcggaacga aactctttga acaatccctt
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 1110

<210> 14
 <211> 369
 <212> PRT
 <213> Acidovorax facilis

<400> 14
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 1 5 10 15
 Pro Val Trp Leu Asp Ala Asp Ala Thr Ile Asp Lys Ser Ile Gly Ile
 20 25 30
 Ile Glu Glu Ala Ala Gln Lys Gly Ala Ser Leu Ile Ala Phe Pro Glu
 35 40 45
 Val Phe Ile Pro Gly Tyr Pro Tyr Trp Ala Trp Leu Gly Asp Val Lys
 50 55 60
 Tyr Ser Leu Ser Phe Thr Ser Arg Tyr His Glu Asn Ser Leu Glu Leu
 65 70 75 80
 Gly Asp Asp Arg Met Arg Arg Leu Gln Leu Ala Ala Arg Arg Asn Lys
 85 90 95
 Ile Ala Leu Val Met Gly Tyr Ser Glu Arg Glu Ala Gly Ser Arg Tyr
 100 105 110
 Leu Ser Gln Val Phe Ile Asp Glu Arg Gly Glu Ile Val Ala Asn Arg
 115 120 125
 Arg Lys Leu Lys Pro Thr His Val Glu Arg Thr Ile Tyr Gly Glu Gly
 130 135 140
 Asn Gly Thr Asp Phe Leu Thr His Asp Phe Ala Phe Gly Arg Val Gly
 145 150 155 160
 Gly Leu Asn Cys Trp Glu His Phe Gln Pro Leu Ser Lys Phe Met Met
 165 170 175
 Tyr Ser Leu Gly Glu Gln Val His Val Ala Ser Trp Pro Ala Met Ser

180					185					190					
Pro	Leu	Gln	Pro	Asp	Val	Phe	Gln	Leu	Ser	Ile	Glu	Ala	Asn	Ala	Thr
		195					200					205			
Val	Thr	Arg	Ser	Tyr	Ala	Ile	Glu	Gly	Gln	Thr	Phe	Val	Leu	Cys	Ser
	210					215					220				
Thr	Gln	Val	Ile	Gly	Pro	Ser	Ala	Ile	Glu	Thr	Phe	Cys	Leu	Asn	Asp
	225					230					235				240
Glu	Gln	Arg	Ala	Leu	Leu	Pro	Gln	Gly	Cys	Gly	Trp	Ala	Arg	Ile	Tyr
			245						250					255	
Gly	Pro	Asp	Gly	Ser	Glu	Leu	Ala	Lys	Pro	Leu	Ala	Glu	Asp	Ala	Glu
			260					265					270		
Gly	Ile	Leu	Tyr	Ala	Glu	Ile	Asp	Leu	Glu	Gln	Ile	Leu	Leu	Ala	Lys
	275					280					285				
Ala	Gly	Ala	Asp	Pro	Val	Gly	His	Tyr	Ser	Arg	Pro	Asp	Val	Leu	Ser
	290					295					300				
Val	Gln	Phe	Asp	Pro	Arg	Asn	His	Thr	Pro	Val	His	Arg	Ile	Gly	Ile
	305					310					315				320
Asp	Gly	Arg	Leu	Asp	Val	Asn	Thr	Arg	Ser	Arg	Val	Glu	Asn	Phe	Arg
			325						330					335	
Leu	Arg	Gln	Ala	Ala	Glu	Gln	Glu	Arg	Gln	Ala	Ser	Lys	Arg	Leu	Gly
			340					345					350		
Thr	Lys	Leu	Phe	Glu	Gln	Ser	Leu	Leu	Ala	Glu	Glu	Pro	Val	Pro	Ala
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Lys

<210> 15
 <211> 1776
 <212> DNA
 <213> Acidovorax delafieldii

<400> 15
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 tttcagtgtg atccacccca aaaatggccc agttttcggt gtgattcaac agcgctcgtg
 120
 ctatgacgtc tactccatac tttcgcaaga aaaagggggg gaaatttttc attccccaat
 180
 tattagggag atcgggtctaa tagtaaaggg caaacctga ttttttatta ggctagatgg
 240
 tctaataatt aaatcagctc ggccaatgcg tagcgctcgg gcaaccaga caaggcaatt
 300
 ctgacagtga caccctctt aggagacgac cgtgggttcg tataacagca agttcctcgc
 360
 ggcaaccgtt caggcagagc cggtatggct cgacgcagac gcaacgatcg acaagtcgat
 420
 cggcatcatc gaagaagctg cccaaaaggg cgcgagtctg atcgctttcc cggaagtatt
 480
 cattccgggc taccctatt gggcgtggct cggcgacgtg aagtacagcc taagctttac
 540

ttcacgctat cacgagaatt cgttggagct aggtgacgac cgtatgcgtc gctccagct
 600
 ggccgcgcgc cgcaacaaaa tcgcactcgt catgggctat tcggagcggg aagccggatc
 660
 gcgctatctg agccaggtgt tcatcgacga gcgtggcgag atcgttgcca a:cggcgcaa
 720
 gctgaagccc acacacgttg agcgtacgat ctacggcgaa ggcaacggaa cggatttcct
 780
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 840
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 900
 gatgtcccct cttcagccgg atgttttcca actgagcacc gaagccaacg cgcaggtcac
 960
 ccgctcgtac gcaatcgaag gccaaacctt tgtgctttgc tcgacgcagg tgatcggacc
 1020
 tagcgcgatc gaaacgttct gcctcaacga cgaacagcgc gcactgttgc cgcgaaggatg
 1080
 tggctgggcg cgcatttacg gcccggatgg aagcgagctt gcgaagcctc tggcggaaga
 1140
 tgctgagggg atcttgtagc cagagatcga tctggagcag attctgctgg cgaaggctgg
 1200
 agccgatccg gtcgggcact attcgcggcc tgacgtgctg tcgggtccagt tcgaccgcg
 1260
 caatcatacg ccagttcatc gcacgcggcat tgacggtcgc ttggatgtga a:accgcgag
 1320
 tcgcgtggag aatttccgac tgcgacaagc ggctgagcag gacgctcagg catccaagcg
 1380
 gctcggaaac aaactctttg aacaatccct tctggctgaa gaaccggctc cagcaaagta
 1440
 gccataagtt gagagtcgcg agatagtatc ggggaaagcc atctctggtc tcccccttta
 1500
 ttctccaagc cgacatcacc gctgaaagcg gggttctttg ctaccccgag ttctgatccc
 1560
 gcacgcgcgt cgcgtgagat ttgcgtcaga gcggacattc aagttgtgtg gaaaggtcgt
 1620
 ccagactgtc cagggaatat tcccagttct cactcggttc aaggtcagtc gtttgcgtcg
 1680
 ggccgtgttc ctgtggccgc ctgacgaatg ccgtcctcag gccacaacgt cgcagcggtg
 1740
 ccaagtcacg gttgtgcgcc gccacatgc agatct
 1776

<210> 16

<211> 1110

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: A synthetic version of the nitrilase gene

<400> 16

atggtttctt acaactccaa gttcttggct gctactgttc aagctgagcc agtttggttg
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 gacgcagacg ctactattga caagtctatc ggtatcatcg aagaagctgc caaaaagggt
 120
 gcctctttga tcgctttccc agaagttttc attccagggt acccatactg ggcttggttg
 180
 ggtgacgtta agtactcttt gtcctttact tccagatata acgagaactc tttggagttg
 240
 ggtgacgaca gaatgcgtag actgcaattg gctgcccgtg gaaacaaaat tgctttggtc
 300

atggggttatt ccgagagaga agctggatct cgttacttgt cccaagtctt catcgacgag
 360
 agaggtgaga ttgttgcaaa tcgtcgttaag ttgaagccaa ctcacgttga gsgtaccatc
 420
 tacggagaag gtaacggaac cgatttcttg actcacgact tcgccttcgg aagagttggt
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 gagcaagtcc acgttgcttc ttggccagct atgtcccctc ttcagccaga tgttttccaa
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 ttgtccatcg aagccaacgc caccgtcacc agatcctacg ccatcgaagg tcaaactttt
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 720
 gaacagagag ctttgttgcc acaaggatgt ggttgggcaa gaatttacgg tccagatgga
 780
 tctgagcttg ccaagccttt ggctgaagat gctgagggta tttgtacgc tgagatcgat
 840
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 900
 gacgtcttgt ccgtccagtt cgaccctaga aaccacactc cagttcacag aattggtatt
 960
 gacggtagat tggatgttaa caccagatcc agagtcgaga acttcagact gagacaagct
 1020
 gctgagcagg agagacaggc ttctaagaga cttggaacta aacttttcga acaatctctt
 1080
 ttggctgaag aacctgtccc agccaagtaa
 1110

<210> 17

<211> 84

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic
oligonucleotide

<400> 17

catgaattca tggtttctta caactccaag ttcttggctg ctactgttca agctgagcca
 60
 gtttggttgg acgcagacgc tact
 84

<210> 18

<211> 90

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic
oligonucleotide

<400> 18

tttgatcgct ttcccagaag ttttcattcc aggttaccca tactgggcct ggttgggtga
 60
 cgtaaagtac tctttgtcct ttacttcag
 90

<210> 19

<211> 90

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Synthetic
 oligonucleotide

 <400> 19
 aattggctgc ccgtagaaac aaaattgctt tggcatggg ttattccgag agagaagctg
 60
 gatctcgta cttgtcccaa gtcttcatcg
 90

 <210> 20
 <211> 90
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Synthetic
 Oligonucleotide

 <400> 20
 gttgagcgtc ccatctacgg agaaggtaac ggaaccgatt tcttgactca cgacttcgcc
 60
 ttcggaagag ttggtggatt gaactgttgg
 90

 <210> 21
 <211> 90
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 21
 agtccacgtt gcttcttggc cagctatgtc ccctcttcag ccagatgttt tccaattgtc
 60
 catcgaagcc aacgccaccg tcaccagatc
 90

 <210> 22
 <211> 90
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 22
 gaccttctgc tatcgaaacc ttctgtctga acgacgaaca gagagctttg tggcacaag
 60
 gatgtgggtg ggcaagaatt tacggtccag
 90

 <210> 23
 <211> 90

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 23
tacgctgaga tcgatttggg gcaaattctg ctggccaagg ctggagccga tccagtcggt
60
cactactcca gacctgacgt cttgtccgtc
90

<210> 24
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 24
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60
gcaggagaga caggcttcta agagacttgg
90

<210> 25
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
oligonucleotide

<400> 25
catgaattct tacttggctg ggacagggtc ttcagccaaa agagattgtt cgaaaagttt
60
agttccaagt ctcttagaag cctg
84

<210> 26
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
oligonucleotide

<400> 26
tgggtgttaac atccaatcta ccgtcaatac caattctgtg aactggagtg tggtttctag
60
ggtcgaactg gacggacaag acgtcaggtc
90

<210> 27
<211> 90

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Synthetic
 oligonucleotide

 <400> 27
 tccaaatcga tctcagcgta caaaataccc tcagcatctt cagccaaagg cttggcaagc
 60
 tcagatccat ctggaccgta aattcttgcc
 90

 <210> 28
 <211> 90
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Synthetic
 oligonucleotide

 <400> 28
 ggtttcgata gcagaaggtc caatgacctg ggtagagcaa aggacaaaag tttgaccttc
 60
 gatggcgtag gatctggtga cggtggcggt
 90

 <210> 29
 <211> 90
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:Synthetic
 oligonucleotide

 <400> 29
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 60
 ggaaatgttc ccaacagttc aatccaccaa
 90

 <210> 30
 <211> 90
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 30
 ccgtagatgg tacgctcaac gtgagttggc ttcaacttac gacgatttgc aacaatctca
 60
 cctctctcgt cgatgaagac ttgggacaag
 90

 <210> 31
 <211> 90

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 31
gtttctacgg gcagccaatt gcagtctacg cattctgtcg tcaccaact ccaaagagtt
60
ctcgtgatat ctggaagtaa aggacaaaga
90

<210> 32
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic
oligonucleotide

<220>

<400> 32
cttctgggaa agcgatcaaa gaggcaccct ttgggcagc ttcttcgatg ataccgatag
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acttgtcaat agtagcgtct gcgtccaacc
90